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Pathogenic variation among isolates of *Rhynchosporium secalis* from barley grass growing in South Eastern Australia

John S. Brown
Department of Agriculture and Rural Affairs, Victorian Crops Research Institute, P.M.B. 260, Horsham 3400, Victoria, Australia

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Summary

The pathogenicity of 182 single spore isolates of *Rhynchosporium secalis* from *Hordeum leporinum* and 94 isolates from *H. vulgare* collected from throughout southeastern Australia was tested on 15 barley varieties, each having different combinations of resistance genes. Forty five percent of the barley grass isolates were pathogenic on 5 or more varieties but only 6% of the cultivated barley isolates attacked this range of varieties. On the basis of reaction type 20 different pathogenicity groups were recognised, with barley grass isolates being classified into 19 and the cultivated barley isolates into 5, four of which were the same as the barley grass isolates. Numerical analysis of data on leaf area damage inferred 33 groups, 24 of which were unique to barley grass isolates, two to cultivated barley isolates and 9 common to both groups. There was as much variation in pathogenicity among single spore isolates from the same lesion as between isolates from different lesions collected from the same or different locations.

Introduction

Knowledge of the pathogenic variability of the fungus is critical to the long term success of a breeding programme to incorporate resistance to *Rhynchosporium secalis* (Oud) J.J. Davis into barley cultivars. Some authors (e.g. Sarasola & Campi, 1947; Houston & Ashworth, 1957; Reed, 1957; Schien, 1960; Kajiwara & Iwata, 1963; Moreno & Vivar, 1975; Ali et al., 1976; Jackson & Webster, 1976; Ceoloni, 1980; Cromey, 1987) recognised distinct pathotypes among the isolates they examined. In contrast to the apparent ease with which these authors were able to differentiate pathotypes, others (e.g. Owen, 1958; Skoropad, 1960; Kline, 1960; Hansen & Magnus, 1973) have not been able to demonstrate such clear-cut distinctions. Nevertheless there is ample evidence that, pathogenically, *R. secalis* is extremely variable even though there is no agreement as to how to interpret this variability.

A major deficiency in previous studies on pathogenic variability of *R. secalis* is that only isolates of the fungus from cultivated barley have been considered. This assumes that the only source of inoculum for the new seasons crop is infected straw from the previous season crop. This is not necessarily the case, as barley grass (*Hordeum leporinum* Link and *H. murinum* L.) represents another extensive source of inoculum. Both are ubiquitous grass weeds in the cereal growing areas of Australia. They are commonly infected with *R. secalis*. Cross inoculation studies (e.g. Owen, 1958; Ali &

Boyd, 1973) have demonstrated that barley grass can be a source of inoculum for scald on cultivated barley.

In a previous study, Brown (1985) divided 319 *R. secalis* isolates from cultivated barley growing in Victoria, Australia, into 5 pathogenicity groups. In that work pathogenic variability among spores from the same scald lesion as well as from different lesions within the same crop was also reported. This paper describes the pathogenic variability among isolates from barley grass and compares it with variation among isolates from cultivated barley.

Materials and methods

Isolate collection. Scald infected barley or barley grass leaves were collected in Tasmania, Victoria, and southern New South Wales between 1980 and 1984. Leaves with fresh lesions were collected from infected plants in barley crops or growing as volunteers on roadsides. Leaf pieces with lesions were surface sterilized in a 1: 1 70% ethanol and 1% NaHClO mixture for 30 sec, rinsed in sterile water, transferred to 1% water agar plates and incubated in continuous light at 15° C for ca. 5 d to encourage sporulation of the fungus. Spores were streaked onto 1% water agar and individual spores transferred to 1% lima bean agar (LBA; Bacto lima bean agar, dehydrated, Difco Laboratories, Detroit) with a micromanipulator equipped with a glass needle.

The study included 182 isolates from barley grass, collected from 42 locations, and 94 isolates from cultivated barley, collected from 32 locations.

Culture storage. To counter the possibility of pathogenicity changes in culture, there was minimal subculturing of isolates between isolation and pathogenicity testing. For long term storage, cultures were covered with sterile liquid paraffin oil and stored in a domestic refrigerator at ca. 5° C. For short term storage, pieces of culture were transferred to sterile culture tubes, covered with sterile water and stored in a domestic refrigerator.

Differential host varieties. The barley varieties used to detect pathogenic variability were selected as including most of the known resistance genes and as having been used in other similar studies. The varieties chosen and their identified genes for resistance are shown in Table 1.

Preparation of inoculum and inoculation. Isolates were subcultured onto LBA and incubated in continuous light at 15° C for 2 wk, after which time spores were harvested by irrigating the culture with water containing 1 drop Tween 20/100 ml. The spore concentration was adjusted to 5×10^4 spores/ml. Seedlings at the 2–3 leaf stage were sprayed with 3 ml/pot of the spore suspension and, after inoculation, were transferred to two controlled environment cabinets each operating at 15(± 1)° C and with a 12 h dark period which commenced when the cabinet was loaded. An automatic mist unit sprayed water onto the seedlings for 5 sec every 25 min to maintain leaf surface wetness.

In all tests, each variety was represented by 5 seedlings growing in a 7 cm diam. pot. Isolates were tested in groups of 26 (13 in each cabinet) and isolates from the same source were included in the same group. After 48 h the seedlings were transferred to the glashouse. Every effort was made to maintain the same conditions for each group of isolates.

Disease assessment. Scald symptoms on the second leaf of each seedling were assessed 21 days after inoculation. Reaction type (RT) was classified on a 0–4 scale where 0 = no visible symptoms, 1 = very small lesions at the tip or on the margin and base of the leaf blade, 2 = small lesions not confined to the leaf margins, 3 = large coalescing lesions covering large areas across the leaf blade and 4 = total collapse of the leaf with no distinct lesions (Ali & Boyd, 1973). Percent leaf area affected (LAA) was visually assessed using standard area diagrams (James et al., 1968) as a guide. For both measures of disease the average of five seedlings was recorded as the reaction of that particular cultivar/isolate combination. Clipper was included in all tests as a standard and if no disease symptoms developed on that cultivar the inoculation was repeated.

Numerical analysis. Numerical taxonomy of the isolates was performed with the NTSYS package (Rohlf, 1985). The basic set of data was the matrix of individual isolates by percent leaf area affected on each of 14 barley cultivars. The Manhattan Metric (Sneath & Sokal, 1973) was used as the measure of dissimilarity between isolates. This was calculated according to the formulae:

$$M = \sum_{k=1}^{s} |X_{ik} - X_{jk}|$$

where X_{ik} and X_{jk} denote the LAA of the k^{th} cultivar affected by the i^{th} and j^{th} isolates and s is the total number of cultivars. The Manhattan Metric was used for hierarchical classification of the isolates by the weighted pair-group method using averages (WPGMA).

Results and discussion

Pathogenicity of R. secalis isolates. In this study an isolate was defined as being pathogenic on a particular cultivar if it incited a response other than a '0' RT or LAA. On this basis all isolates tested were pathogenic on Clipper (Table 2). CI 3515 was the only cultivar not affected by at least one barley grass isolate (Table 2). There were no cultivated barley isolates pathogenic on Atlas 46, Hudson, Osiris, CI 3515 or CI 4364 (Table 2). A greater proportion of the barley grass than the cultivated barley isolates was pathogenic on Wisconson Winter × Glabron, Turk and Modoc; Atlas was attacked by a greater proportion of the cultivated barley isolates (Table 2). Because no isolates were pathogenic on CI 3515, this cultivar was excluded from the data matrix that was used for pathotype identification.

With the exception of six barley grass isolates, those tested were pathogenic on more than one cultivar. The maximum number of cultivars affected by any one barley grass isolate was eight; for the cultivated barley isolates the figure was five. For barley grass isolates the most common number of cultivars affected by any one isolate was five; it was three for the cultivated barley isolates. Forty-four percent of the barley grass isolates were pathogenic on five or more cultivars compared with only 6% of the cultivated barley isolates. Thus, the barley grass isolates were apparently pathogenically more diverse than the isolates from cultivated barley.

Table 1. Barley varieties used to detect pathogenic variability in *R. secalis*

Cultivar	CI No.	Resistance genes
Abbyssinian	668	Rh9[a]
Atlas	4118	Rh2[b]
Atlas 46	7323	Rh Rh2[b]
Brier	7157	Rh rh6[b]
Hudson	8067	Rh[b]
La Mesita	7565	Rh4 Rh10[b]
Modoc	7566	Rh2 rh6[b]
Nigrinudum	2222	rh8[b]
Osiris	1622	Rh4 Rh10 rh6[b]
Stuedelli	2266	rh6 rh7[a]
Turk	14400	Rh rh6 Rh5[b]
Wisconsin Winter × Glabron	8162	Rh3[b]
unnamed	3515	Rh4 Rh10[b]
unnamed	4364	rh11[b]
Clipper	14844	–

[a] Baker & Larter (1963).
[b] Habgood & Haynes (1971).

Table 2. Number and percentage of isolates of *R. secalis* from barley grass and from cultivated barley that were pathogenic on each test cultivar (i.e. the isolated incited a response other than '0' RT or LAA)

Cultivar	Barley grass		Cult. barley	
	No.	%	No.	%
Abbyssinian	4	2	1	1
Atlas	2	1	32	34
Atlas 46	1	1	0	0
Brier	171	94	90	96
Hudson	4	2	0	0
La Mesita	12	7	7	7
Modoc	35	19	4	4
Nigrinudum	5	3	2	2
Osiris	1	1	0	0
Stuedelli	164	90	93	99
Turk	74	41	2	2
Wisconsin Winter × Glabron	117	64	2	2
CI 3515	0	0	0	0
CI 4364	1	1	0	0
Clipper	182	100	94	100

However, this may not be a real difference. Sample size may account for some of the difference as there were twice as many barley grass as cultivated barley isolates. Many of the cultivars affected only by isolates from barley grass were affected by so few isolates that a larger sample from cultivated barley may have identified isolates pathogenic on these cultivars.

Grouping isolates using reaction type. Isolates producing RT '0–2' were classified as avirulent and those producing RT '3–4' as virulent. On this basis 20 different pathogenicity groups were recognised, with the barley grass isolates being classified into 19 and the cultivated barley isolates into five, four of which were the same as those of the barley grass isolates (Table 3). This study recognised the same five pathogenicity groups among the cultivated barley isolates that were reported by Brown (1985). Of the other published studies, only Ali et al. (1976), Jackson & Webster (1976) and Cromey (1987) included comparable numbers of isolates (203, 175 and 149 respectively). The present findings concurs with the conclusion of Ali et al. (1976) and Jackson & Webster (1976) that there appears to be many pathotypes of the fungus. Cromey (1987) concluded that there were only four pathotypes.

This study has revealed that some cultivars which have been reported to have common genes for resistance, did not react identically. Hudson is reported to carry only the *Rh* gene (Habgood & Hayes, 1971) whereas Turk & Brier carry *Rh* in addition to other genes (Habgood & Hayes, 1971). One would therefore expect that isolates that were virulent on Turk and/or Brier would also be virulent on Hudson. However, isolates were identified that were virulent on Turk and/or Brier but avirulent on Hudson. Similarly, on the basis of identified resistance genes, isolates that were virulent on

Table 3. The twenty pathogenicity groups derived from classification of isolates of *R. secalis* (182 from barley grass [BG] and 94 from cultivated barley [CB]) using reaction type as the criterion

Group	Virulence on differential varieties													No. isolates	
	Abby	Atla	At46	Brie	Huds	LaMe	Modo	Nigr	Osir	Stue	Turk	WiWG 4364	Clip	CB	BG
I													+	26	54
II		+											+	1	
III										+			+	4	11
IV				+									+	9	20
V				+						+			+	54	31
VI											+		+		9
VII				+							+		+		9
VIII											+	+	+		1
IX										+	+		+		1
X				+			+						+		1
XI				+								+	+		2
XII										+		+	+		1
XIII				+							+	+	+		1
XIV				+						+	+		+		5
XV				+						+	+	+	+		21
XVI				+		+				+	+		+		1
XVII				+						+	+	+	+		7
XVIII				+		+				+		+	+		2
XIX				+		+	+			+		+	+		2
XX															3
No. groups														5	19

+ = Virulent Reaction (RT ≥ 3)

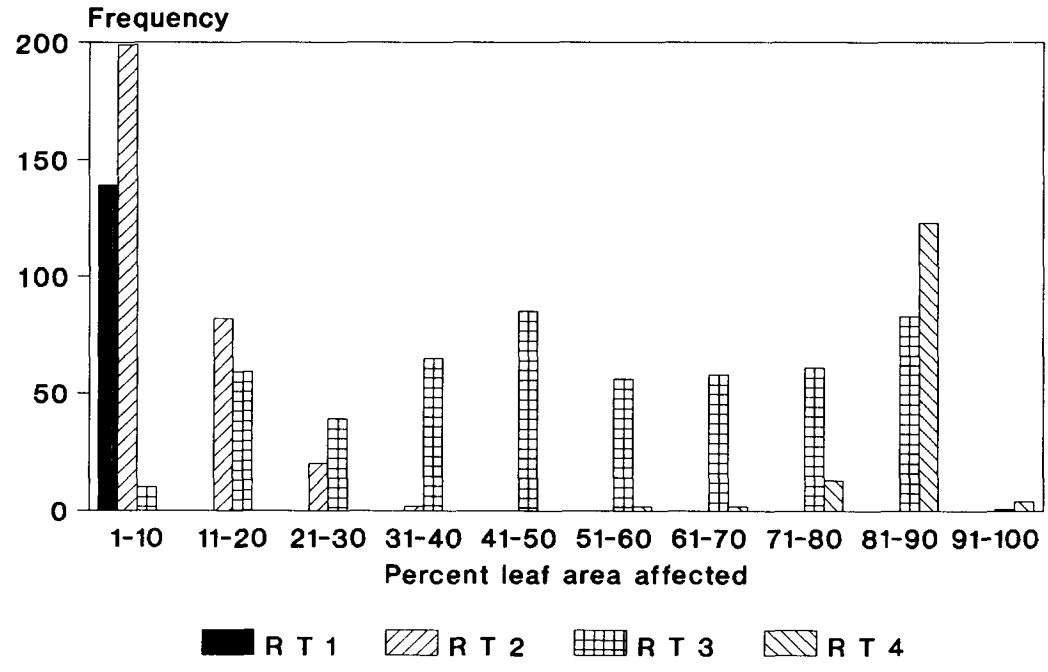

Fig. 1. Relationship between reaction type and percent leaf area affected for infection of barley cultivars by isolates of *R. secalis*.

Turk would also be expected to be virulent on Brier; isolates virulent on La Mesita should be virulent on CI 3515; isolates virulent on both Atlas and Brier should be virulent on Atlas 46 and isolates virulent on La Masita and any one of Brier, Turk or Modoc should also be virulent on Osiris. Isolates that did not behave as predicted above were identified in these studies. In addition, Clipper is not reported to carry any genes for resistance to *R. secalis,* but some barley grass isolates (Group XX, Table 3) that were pathogenic on this cultivar were classified as avirulent. Similar discrepancies have been reported by others (Jackson, 1975; Ceoloni, 1980; Cromey, 1987). They are probably due to either unrecognised differences in the genes described or to the presence of previously undetected genes for resistance/genes for avirulence. Either hypothesis is acceptable and can be applied to explain the reactions of Hudson, Brier, Atlas 46, CI 3515, Orisis and Clipper in this study.

Relationship between reaction type and leaf area affected. Traditionally, virulence in *R. secalis* has been measured in terms of RT. However, a closer examination of the data presented in this study suggests that RT may not be an appropriate criterion. RT 1 was always associated with low LAA, and RT 4 with a high LAA; RT 2 tended to be associated with low LAA, but RT 3 was associated with a wide range of LAA, from low to high (Fig. 1). This suggests that it may not be appropriate to make the split between virulence and avirulence between RT 2 and RT 3. There are no published comparisons of the RT and LAA measures of the isolate/cultivar interaction but LAA is a much more appropriate measure of disease since damage is related more to area of leaf affected than to the types of lesion (James & Teng, 1979).

Nevertheless the problem of how to make the distinction between avirulence and virulence still remains. Williams & Owen (1973) reported using percent leaf area damaged as a measure of virulence of *R. secalis*. They defined a virulent isolate as one which caused disease symptoms affecting more than 10% of the leaf area. However there was no obvious discontinuity in the data recorded in the present study, although there was a trend to fewer records in the 20–30% categories.

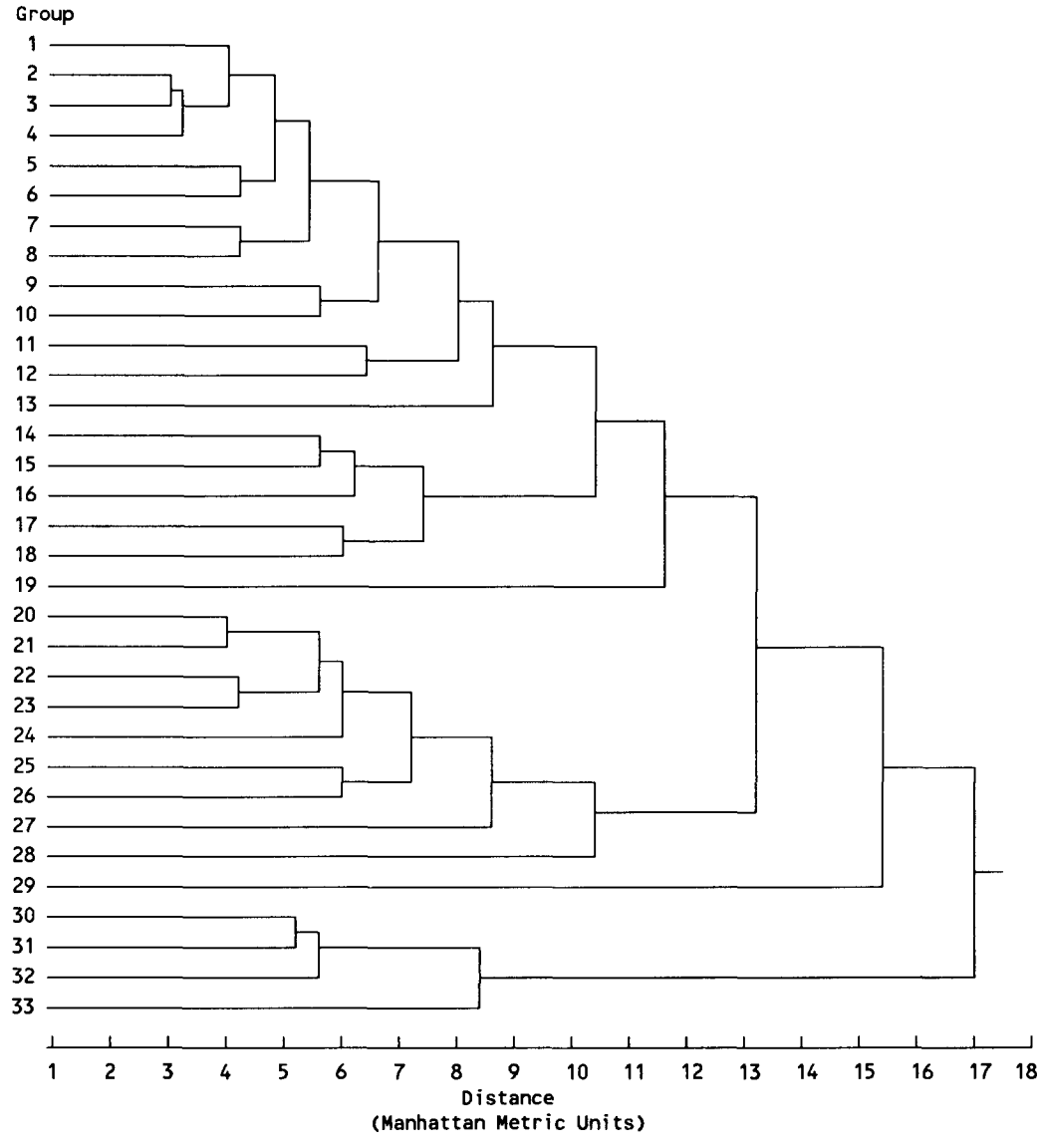

Fig. 2. Hierarchical classification of 276 isolates of *R. secalis* by the weighted pair-group method using averages based on percent leaf area affected on 14 barley cultivars.

Grouping isolates using numerical analysis. Numerical analysis offers a procedure for grouping isolates that is able to take into account the quantitative nature of the isolate/cultivar interactions. The WPGMA method of clustering produced distinct clusters with large differences between fusion levels and an arbitrary merging of clusters at distances less than 3.0 Manhattan Metric units produced groups of isolates that were, pathogenically, homogeneous. The truncated dendrogram resulting from the WPGMA method is illustrated in Fig. 2 and the pathogenicity characteristics of each of the resulting 33 groups are summarised in Table 4. The barley grass isolates were classified into 32 and cultivated barley isolates into 11 groups. That 11 of the groups consisted of a single isolate and 21 groups had five or less members clearly illustrates

the extensive pathogenic variability of *R. secalis*, particularly among isolates from barley grass.

This contrasts with the 20 groups that were recognised when RT was used as the basis of classification (Table 3) and reinforces the conclusion that RT may not be appropriate because it masks the quantitative nature of the host/pathogen interaction, thereby providing a false impression of the pathogenic variability of the fungus. Furthermore, by taking a selected sample of the isolates used in this study it would be possible to demonstrate the existence of definite clusters and hence infer the existence of a limited range of pathotypes. This highlights the danger of considering only a limited sample of isolates in any study of pathogenic variability and may be the reason why some researchers concluded that there were distinct pathotypes of *R. secalis*.

Pathogenic variability within and between locations. The extreme nature of the variation in pathogenicity of *R. secalis* was further exemplified by the

Table 4. Pathogenicity characteristics (mean percent leaf area affected) of the 33 groups identified by hierarchical classification

Group	Mean percent leaf area affected on differential cultivars														No. isolates	
	Abby	Atla	At46	Brie	Huds	LaMe	Modo	Nigr	Osir	Stue	Turk	WiWG	4364	Clip	CB	BG
1	0	0	0	45	0	0	0	0	0	38	0	0	0	84	18	4
2	0	0	0	22	0	0	0	0	0	30	0	0	0	81	8	1
3	0	0	0	0	0	0	0	0	0	40	0	0	0	80	1	1
4	0	0	0	12	0	0	0	0	0	45	0	1	0	91	4	9
5	0	0	0	58	0	0	0	0	0	66	0	0	0	86	22	4
6	0	0	0	60	0	0	1	0	0	54	1	20	0	89		16
7	0	0	0	20	0	0	0	0	0	60	0	23	0	95		2
8	0	0	0	30	0	20	0	0	0	50	0	20	0	80		1
9	0	0	0	60	0	3	3	0	0	50	3	25	0	60		2
10	0	0	0	50	0	10	30	0	0	50	10	25	0	80		1
11	0	0	0	0	0	0	0	0	0	50	0	50	0	90		1
12	0	0	0	45	0	0	0	0	0	55	30	60	0	90		2
13	2	0	1	50	0	0	12	8	0	21	13	24	0	90		7
14	0	2	0	76	0	0	0	0	0	86	0	2	0	88	13	3
15	0	0	0	70	0	0	0	0	0	80	50	20	0	90		1
16	0	0	0	86	0	5	8	0	0	60	13	27	0	90		5
17	0	0	0	73	0	0	1	0	0	69	1	48	0	91		9
18	0	0	0	90	0	0	0	0	0	90	0	80	0	90		1
19	0	0	0	67	0	22	37	0	0	63	25	57	0	90		3
20	0	0	0	11	0	0	1	0	0	6	5	4	0	88	16	51
21	0	0	0	43	0	0	0	0	0	11	7	5	0	90	2	7
22	0	0	0	13	0	0	0	0	0	5	40	7	0	91		4
23	0	0	0	21	0	0	0	0	0	13	67	10	0	90		7
24	0	0	0	10	0	0	0	0	0	40	20	0	0	100		1
25	0	0	0	27	0	0	0	0	0	30	50	33	0	90		3
26	0	0	0	30	0	0	0	0	0	60	60	0	0	90		1
27	0	0	0	55	0	0	0	0	0	13	85	10	0	90		2
28	0	5	0	70	0	0	0	0	0	20	60	80	0	90		1
29	0	0	0	90	0	90	0	0	0	30	20	10	0	90		1
30	0	0	0	7	0	0	0	0	0	4	0	1	0	49	8	28
31	0	0	0	10	0	0	0	0	0	20	0	30	0	70		1
32	0	0	0	43	0	0	0	0	0	14	0	8	0	50	1	2
33	0	50	0	20	0	0	0	0	0	5	0	0	0	80	1	
No. groups															11	32

finding that spores from the same lesion, as well as spores from different lesions collected at the same location, were pathogenically very variable and were classified into different pathogenicity groups by both qualitative and quantitative methods.

At 10 locations from where diseased leaves were collected, isolates were obtained from each of three separate lesions. For two locations, the three isolates were all classified into the same pathogenicity group by numerical analysis. For 6 locations the isolates were classified into two groups and for the remaining two locations each of the isolates were classified into separate groups (Table 5).

Multiple single spore isolates were obtained from a single lesion at each of seven locations. Five isolates were obtained from each of four lesions. For lesion 128.1 the five isolates were all classified into the same pathogenicity group by numerical analysis; for lesion 112.1 the isolates were classified into two groups and isolates from lesions 100.2 and 126.1 were each classified into three groups, the eight isolates from lesion 144.1 were classified into three groups; the eight isolates from 144.1 into four groups, and the eight isolates from 145.1 into 6 groups. The 23 isolates from lesion 141.2 were classified into nine pathogenicity groups (Table 6). Variation in pathogenicity among single sopre isolates of *R. secalis* from the same source has also been reported by Habgood (1973), Hansen & Magnus (1973) and Jackson & Webster (1976). The last authors were the only ones to describe the variation as pathotypes.

The *R. secalis* population on barley grass may reasonably be expected to be pathogenically the more diverse than that on cultivated barley since the barley grown in Australia is derived from a limited number of English cultivars (Ali et al., 1976). By contrast, the barley grass populations have been shown to be quite diverse (Cocks et al., 1976; Ali, 1981). The present results support this hypothesis since they indicate that individual barley isolates possess a greater range of pathogenic abilities than individual cultivated barley isolates. Further, this study has confirmed that barley grass is an important source of inoculum of *R. secalis* for barley crops because all isolates from barley grass affected at least one barley cultivar. This source of scald variants must, therefore, be considered when barley cultivars resistant to the disease are being developed.

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Table 5. Pathogenic variability among isolates of *R. secalis* from three different lesions collected from the same location

Location	Pathogenicity group[2] (Number of isolates[3])		
99	2 (1)	4 (2)	
100	20 (2)	21 (1)	
102	20 (3)		
106	7 (1)	16 (2)	
107	20 (1)	21 (1)	30 (1)
115	6 (1)	15 (1)	16 (1)
125	1 (1)	30 (2)	
126	6 (2)	31 (1)	
128	30 (3)		
130	1 (2)	30 (1)	

[2] According to hierarchical cluster analysis (Fig. 2, Table 4).
[3] One isolate was taken from each of three lesions at each location.

Table 6. Pathogenic variability among isolates of *R. secalis* from the same lesion

Lesion	No. isolates derived from each lesion	Pathogenicity group[1] (No. isolates[2])			
100.2	5	20 (2)	21 (1)	22 (2)	
112.1	5	20 (4)	30 (1)		
126.1	5	6 (3)	17 (1)	31 (1)	
128.1		30 (5)			
141.2	23	3 (1)	4 (3)	16 (1)	20 (9)
		21 (1)	22 (1)	23 (3)	25 (3)
		26 (1)			
144.1	8	1 (1)	6 (2)	14 (2)	17 (3)
145.1	8	12 (2)	13 (1)	17 (1)	20 (1)
		21 (2)	28 (1)		

[1] According to hierarchical cluster analysis (Fig. 2, Table 4).
[2] The number of isolates in each group.

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